

FIG. 1

Figure 1. Process Flow Chart

The cells are resuspended in buffer using a static mixer and a closed vessel. Cell lysis is completed by a modification of the alkaline lysis method followed by neutralization. Gentle mixing is completed using a static mixer in continuous re-circulation mode.

Removal of cell debris and clarification is accomplished by diatomite aided depth filtration.

After clarification, the lysate is concentrated 10-15 fold using hollow fiber ultrafiltration followed by a buffer exchange.

The concentrated nucleic acid is selectively precipitated using PEG for further enrichment and concentration.

The PEG pellet is collected by centrifugation and dissolved in buffer. Ammonium acetate is added to the solution to selectively precipitate contaminants which are removed by centrifugation. The supernatant is IPA precipitated and stored at -20 until chromatography step. STOP POINT

The IPA precipitated nucleic acid is pelleted by centrifugation. The pellet is dissolved in column buffer A, 0.22um filtered and loaded onto the column. After loading there are intermediate step washes before product elution.

The HyperD elution peak is diluted 1:1 with 3M ammonium sulfate, 0.22um filtered then loaded onto the HIC column. Under the load conditions product flows through in the void volume; residual contaminants bind to the column.

The purified pDNA is concentrated followed by a buffer exchange to remove the ammonium acetate.

The purified DNA is ethanol precipitated and stored at -20 C until to bulking.

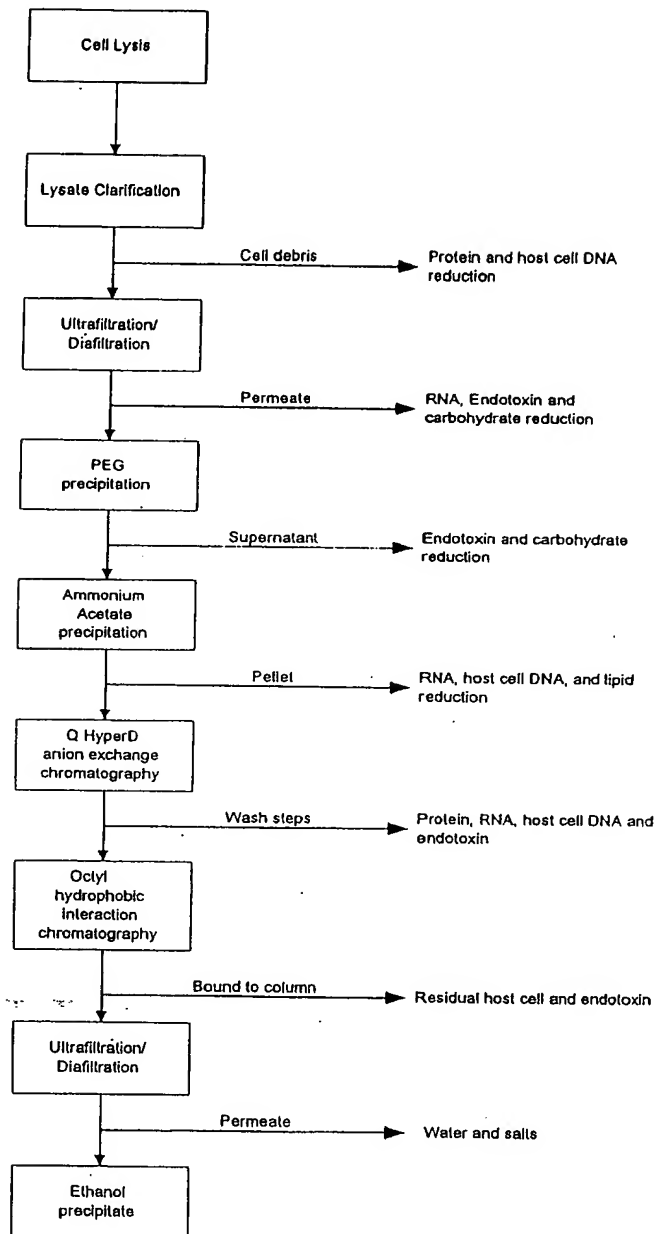


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